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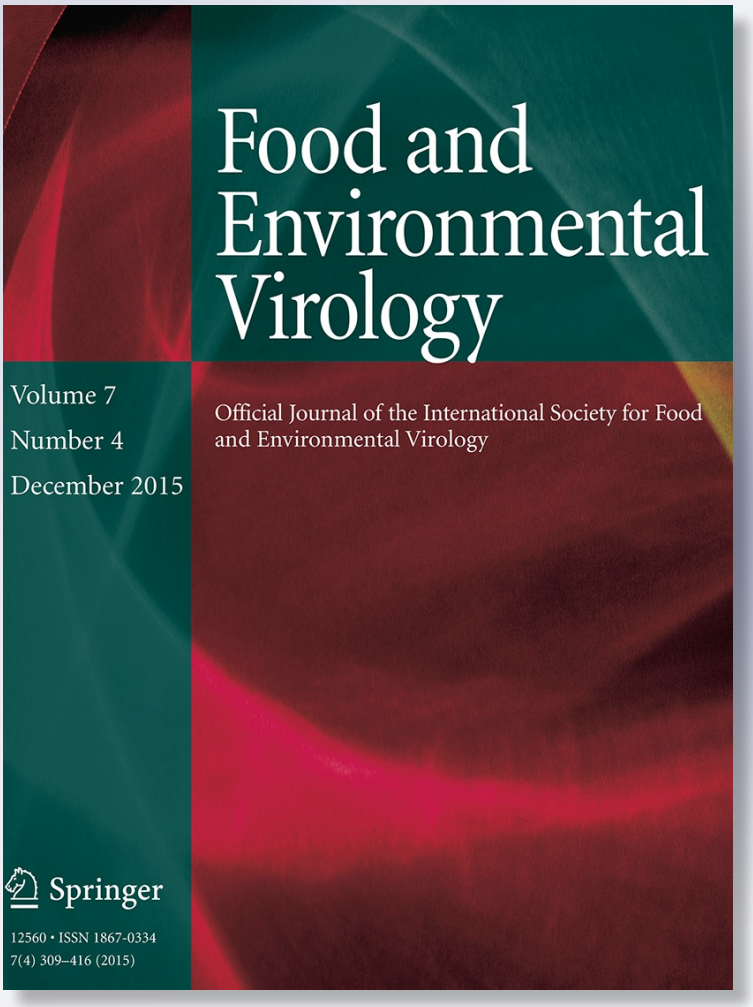

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ORIGINAL PAPER

Application of F$^+$RNA Coliphages as Source Tracking Enteric Viruses on Parsley and Leek Using RT-PCR

Dina Shahrampour[1] · Masoud Yavarmanesh[1] · Mohammad Bagher Habibi Najafi[1] · Mohebbat Mohebbi[1]

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Abstract The objective of this study was to identify sources of fecal contamination in leek and parsley, by using four different F$^+$RNA coliphage genogroups (IV, I indicate animal fecal contamination and II, III indicate human fecal contamination). Three different concentrations (10^2, 10^4, 10^6 pfu/ml) of MS2 coliphage were inoculated on the surface of parsley and leek samples for detection of phage recovery efficiency among two methods of elution concentration (PEG-precipitation and Ultracentrifugation) by performing double agar layer (DAL) assay in three replications. Highest recovery of MS2 was observed in PEG method and in 10^6 inoculation concentration. Accordingly, the PEG method was used for washing and isolation of potentially contaminated phages of 30 collected samples (15 samples from the market and 15 samples from the farm). The final solutions of PEG method were tested for the enumeration of plaques by DAL assay. Total RNA was then extracted from recovered phages, and RT-PCR was performed by using four primer sets I, II, III, and IV. Incidence of F$^+$RNA coliphages was observed in 12/15 (80 %) and 10/15 (66/6 %) of samples were obtained from farm and market, respectively, using both DAL and RT-PCR test methods. Different genotypes (I, II, and IV) of F$^+$RNA coliphages were found in farm samples, while only genotype I was detected in market samples by using the primer sets. Due to the higher frequency of genotype I and IV, the absence of genotype III, and also the low frequency of genotype II, it is concluded that the contamination of vegetable (parsley and leek) in Neyshabour, Iran is most likely originated from animal sources.

Keywords Parsley and leek · Microbial source tracking · F$^+$RNA coliphage · PEG-precipitation · Ultracentrifugation · RT-PCR

Introduction

Fecal contamination of food and water resources is a serious problem for human health. Identification of fecal pollution sources plays a significant role in management and interception strategies.

Fecal material contains different pathogenic microorganisms, including viruses. Since viruses cannot replicate on environmental and food surfaces, so their presence on vegetables are the results of fecal contamination. The contamination of fresh product can occur in farm by polluted water and sewage sludge used for irrigation and fertilization or by handling of infected person with poor hygiene (Bidawid et al. 2000; Seymour and Appleton 2001; D'Souza et al. 2006; Dubois et al. 2006; Fumian et al. 2009). In recent years, consumption of raw or minimally processed vegetables has been increased because of their health effect. Also, some reports implicated on outbreak of food borne viral infections such as gastroenteritis and hepatitis after use of fresh products (Pebody et al. 1998; Schwab et al. 2000; Long et al. 2002; Koopmans and Duizer 2004; Heaton and Jones 2008; Kokkinos et al. 2012). So, in addition to shellfish and fruits, raw vegetables have been recognized as a vehicle for virus transmission (Butot et al. 2007; Croci et al. 2008).

Unfortunately, some studies indicated that conventional household washing to remove pathogenic enteric viruses

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from vegetables surfaces was unsuccessful (Croci et al. 2002; Dubois et al. 2002). Due to low concentration of viruses in food products, methods with high sensitivity such as molecular techniques are essential for detection of these organisms (Papafragkou et al. 2008; Stals et al. 2012). Although numerous protocols for detecting enteric viruses in different fruits and vegetables have been published, but standard and valid virological methods are not available (Dubois et al. 2006). On the other hand, culturing of important food borne viruses like noroviruses (NoVs) and hepatitis A (HAVs) in laboratory has been impossible so far (Koopmans and Duizer 2004). However, no direct correlation between traditional indicators like fecal coliform and enteric viruses has been claimed (Sundram et al. 2002; Abbaszadegan et al. 2003). Therefore, some studies have proposed bacteriophages as an ideal viral and fecal indicator (Sundram et al. 2002; Abbaszadegan et al. 2003; Grabow 2004; Rahman et al. 2009). Currently, male-specific F^+RNA bacteriophages have received the most attention as surrogate and model for enteric viruses in several researches because of similarity to mammalian viral pathogens in shape, size, morphology, isoelectric point, resistant to environmental stress, and transport characteristics (Grabow 2004; Jofre et al. 2011; Mesquita and Emelko 2012). These coliphages infect gram-negative bacterial cells that produce F or sex-pili (i.e., *Escherichia coli K12* and *Salmonella typhimurium WG49*) and unlike other viral and bacterial indicators, working with them in laboratory are easy, inexpensive and also they have little risk to human health (Havelaar and Hogeboom 1984; Debartolomeis and Cabelli 1991; Grabow 1998).

These coliphages are divided into four different subgroups, which group II and III are generally associated with human sources of fecal contamination, and group I and IV are generally associated with animal wastes. Therefore, one of the valuable functions of F^+RNA coliphages is to discriminate human from non-human fecal contamination in microbial source tracking studies that can be carried out with serotyping or genotyping of F^+RNA coliphages isolates (Hsu et al. 1995; Schaper and Jofre 2000; Cole et al. 2003; Noble et al. 2003; Lee et al. 2009). Recently, these coliphages have been used as a good tool for microbial source tracking in waters (Sobsey et al. 2006; Stewart-Pullaro et al. 2006; Gourmelon et al. 2007; Kirs and Smith 2007; Gourmelon et al. 2010).

The presence of F^+RNA coliphages has been reported not only in polluted water but also in different food stuffs such shellfish, poultry, salad vegetables, parsley, and cilantro (Hsu et al. 2002; Endley et al. 2003; Muniain-Mujika et al. 2003; Williams 2005; Kirs and Smith 2007; Wolf et al. 2008). Microbial source tracking approach is believed to be a useful tool in application of F^+RNA coliphages. The purpose of this study was to apply simple methods for enumeration and genotyping of F^+RNA coliphages to identify sources of fecal contamination in blind parsley and leek samples obtained from farm and market.

Materials and Methods

Sample Collection

Thirty parsley and leek samples were collected from three suspected farms (15 samples) and three markets (15 samples) located in Neyshabour city, North East of Iran, from April to July 2013 (spring to summer). The farms (A, B, and C) were in the suburbs. It was possible that some animals (cats and dogs) and birds (chickens and crows) crossed in these farms. Moreover, fertilization with animal faces (hens and cows) and irrigation with groundwater was carried out by farmers. Two markets A and C were in city center, but market B with lower hygiene was in a different site of Neyshabour city.

All collected samples were stored in sterile plastic bags at 4 °C and analyzed within 24 h.

Evaluation of Extraction and Concentration Methods for Phages Recovery

Inoculation of Vegetable Samples

MS2 coliphages (ATCC 15597-B1) were selected as surrogate for male-specific F^+RNA coliphages in phage recovery analysis. MS2 stocks were tittered using the double agar layer (DAL) method (USEPA 2001). Viral stock was diluted in phosphate-buffered solution (pH 7.2) to achieve the following concentrations: 3.4×10^6, 3.4×10^4, and 3.4×10^2 pfu/ml. Aliquots (100 µl) of every dilution were distributed on the surface of three samples of parsley and leek which were mixed. Each sample was placed in a polypropylene filter bag (Interscience, France) and left to dry for 30 min at room temperature under a laminar hood before starting sample processing. Un-inoculated vegetable sample was used as negative control in each test. Following methods subsequently performed for choosing the best methods for phage recovery.

Polyethylene Glycol Precipitation

The method of Scherer et al. (2010) with slight modifications was used. Briefly, 10 g of inoculated mixture of parsley and leek were rinsed with 40 ml elution buffer (100 mM Tris–HCl, 50 mM glycine, 1 % beef extract, pH 9.5), for 20 min at room temperature by gentle shaking. The rinse fluid was removed via bag filtration and centrifuged at 10,000×*g* for 30 min at 4 °C to sediment residual food

particles. The pH of the supernatant was adjusted to 7.2 ± 0.2 by addition of 5 N HCl. The neutralized fluid was supplemented with 0.25 volumes of a 50 % polyethylene glycol 8000 (PEG; Sigma-Aldrich, Germany) in 1.5 M NaCl, followed by incubation for 4 h at 4 °C. Phages were then concentrated by centrifugation at 10,000×*g* for 30 min at 4 °C. The pellet was resuspended in 500 µl of PBS and used in next steps (Fig. 1).

Ultracentrifugation

The method was performed as described by Rzezutka et al. (2005) for a vegetable sample size of 25 g. Mixture of 50 ml of 1 M $NaHCO_3$ buffer with 1 % soy protein powder (90 % soy protein) and 500 µl of Catfloc T (Calgon Corp., USA) which was diluted to 34.5 % solution in Tris–glycine (100 mM Tris, 50 mM glycine) buffer were added to each sample. Ultracentrifugation was done at 40,000×*g* for 4 h. The pellet was resuspended in PBS after the supernatant was decanted and used in next steps (Fig. 1).

Coliphages Enrichment

The phage solution which was prepared in the previous step of markets and farms vegetables samples was enriched to obtain better phage identification in the molecular technique. The phage solution was centrifuged at 8000×*g*, for 10 min and the clarified supernatant passed through a 0.22-µm filter to remove any endogenous bacteria. Then, 1 ml of filtered sample was added to 4 ml of sterile broth medium (TSB) in a sterilized tube, inoculate with 100 µl of a fresh overnight host culture (*E. coli* F_{amp}). Enrichment culture was incubated at 37 °C overnight and then stored at 4 °C until genotyped (Center for Phage Technology Texas 2011).

Enumeration of F-Coliphages

Suspension made from PEG-precipitation and Ultracentrifugation methods was assayed for MS2 coliphage enumeration in inoculation samples by DAL technique (USEPA, 2001). *Escherichia coli* F_{amp} (ATCC 700891) was used as host bacteria for male-specific coliphages. Briefly, in this technique, 500 µl of phage suspension followed by 100 µl host suspension was added to 5 ml tryptic soy agar (TSA) containing 0.7 % agar and 50 µl antibiotic (ampicillin and streptomycin) as top agar layer and then poured onto bottom TSA plate. Each sample was analyzed by DAL assay in duplicate. Also each inoculation experiment was tested in triplicate. The plates were incubated at

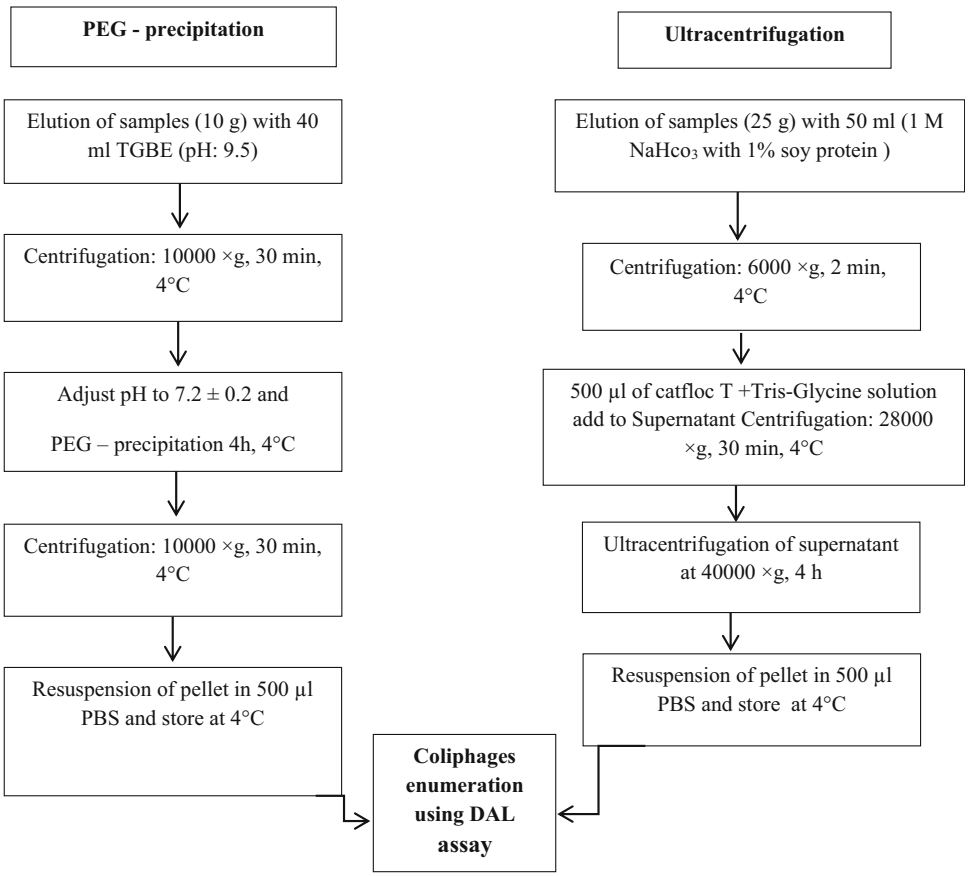

Fig. 1 Flowchart for recovery and concentration of coliphages in parsley and leek samples

37 °C overnight, and after that clear zones (plaques) were counted. Positive and negative controls were included in each test. The numbers of plaques per each plate were recorded to determine the percent of MS2 coliphage recovery for each extraction and concentration methods and choose the best one for blind samples (Table 2).

About blind parsley and leek samples that collected from various farms and markets, after recovery of coliphages, the DAL assay was carried out and plaques were counted too. Then, plaques were dissolved in 5 ml of sterilized PBS (pH 7.4) that poured on the surface of culture plates for 60 min and after filtration (pore size 0.2 μm) solution stored at 4 °C until molecular techniques performed.

RNA Extraction

The heat-release procedure was applied for RNA extraction of viral enrichment solution. For the undiluted sample, 10 μl of coliphage supernatant was heated in a thin-walled 250 μl PCR tubes for 5 min at 98 °C and chilled on ice for 2 min (Vinjé et al. 2004; Friedman et al. 2009). Aliquots of 1 μl were immediately applied into the RT-PCR reaction. Purified viral RNA from representative phage strains was used as a viral RNA control.

RT-PCR

Extracted RNA was transcribed into cDNA via RT kit (Fermentas, Canada) according to the manufacturer's instruction. Then, for each 25 μl PCR reaction volume, 1 μl of synthesized cDNA, 1 μl of primer, and 10.5 μl of RNase-free sterilized water were added to 12.5 μl of Master mix red solution containing PCR reaction buffer [$MgCl_2$, dNTP, and DNA polymerase (*Taq*) enzyme]. The PCR reaction was performed using the Thermo cycler device (Sensequest, Germany) with such conditions: 95 °C for 15 min followed by 40 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min with a final extension of 72 °C for 10 min. Amplicons were separated by gel electrophoresis in 2 % agarose, stained with DNA green viewer, and visualized under UV light (Geldoc, Sony, Japan). For each reaction, a positive (specified template RT-PCR for F^+RNA coliphages) and negative control (no-template RT-PCR) was prepared. To avoid contamination, PCR master mixes, amplification, electrophoresis, and template and/or viral preparations were conducted in separated room. Sequencing of PCR products was carried out by Macrogen Company (Macrogen, Korea).

Primers

Primers depicted in Table 1 were synthesized by Macrogen Company (Macrogen, Korea) and used in RT-PCR for detection of four groups of F^+RNA coliphages.

Statistical Analysis

Results were reported as the average of three replications (all treatments were evaluated in three batches). In order to assess significant differences among different detection methods and among inoculation concentration, a complete randomized design of triplicate analyses of samples was performed using the Minitab 16.0.2 (Minitab Inc., State College, PA, USA) software. Duncan's new multiple range tests were used to study the statistical differences of the means with 95 % confidence.

Results

Recovery Efficiency of MS2 Coliphage in PEG-Precipitation and Ultracentrifugation Methods

Results showed that MS2 coliphages were successfully recovered in the both methods. However, higher recovery efficiency was observed by PEG-precipitation method in three spiked solutions of MS2 coliphage. Also statistical significant difference was found between two methods in the last spiked solution (3.4×10^6 pfu/ml) with 95 %

Table 1 Sequence of primers for F^+RNA coliphages

FRNA coliphages group	Primer	Sequence	Molten temperature	Amplicon (bp)	Source	Ref
I	Forward	5′-AATCTTCGTAAAACGTTCGTGTC-3′	58	204	Non-human	Dryden et al. (2006)
	Reverse	5′-GAGCCGTACCCACACCTTATAG-3′	59.8			
II	Forward	5′-CGTACTTAGCGGTATACTCAAGACC-3′	60.56	240	Human	Dryden et al. (2006)
	Reverse	5'-GTTTCCTGCATATAAGCATACCA-3'	56.72			
III	Forward	5′-AAG AAC AGT AAAG ACAT GAT CG -3′	54.26	229	Human	Vinjé et al. (2004)
	Reverse	5′-CCC ATGAT GAAG GAAT ATCT TTC TC-3′	57.32			
IV	Forward	5′-TTAAACTAATTGGCGAGTCTGTACC-3′	59	236	Non-human	Dryden et al. (2006)
	Reverse	5′-AACAGTGACTGCTTTATTTGAAGTG-3′	58.5			

Table 2 Recovery efficiency of MS2 coliphages from spiked samples (parsley and leek)

Method	Spiked samples (pfu/ml)	Recovery (pfu/g)	Recovery efficiency (%)
PEG-precipitation	3.4×10^2	1.7×10	5
	3.4×10^4	2.16×10^3	6.35
	3.4×10^6	4.08×10^5	12
Ultracentrifugation	3.4×10^2	1.2×10	3.5
	3.4×10^4	1.78×10^3	5.23
	3.4×10^6	2.86×10^5	8.4

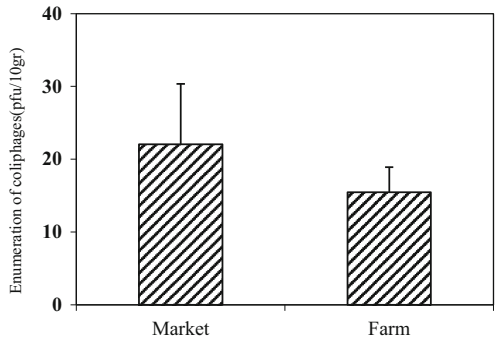

Fig. 2 Enumeration comparison of coliphages in farm and market samples

confidence (Table 2). Therefore, PEG-precipitation method was chosen for evaluating blind parsley and leek samples.

Enumeration of Male-Specific Coliphages in Different Samples Using Plaque Assay

Twenty-two samples of thirty mixed parsley and leek samples were male-specific coliphages positive. Twelve of these positive samples were from farms and the remaining ten were from markets. Enumeration of coliphages was <10 pfu/10 g in 40 and 33.3 % of farm and market samples, respectively, whereas this enumeration was 10–50 pfu/10 g in the rest vegetable samples. Generally, enumeration of coliphages in market samples was slightly more than farm, but no statistical significant difference was observed between them (Fig. 2).

The highest enumeration of coliphages was in market B, whereas it was the lowest in market A. Also, statistical significant difference was observed among market B and another sample collection sites. Similar enumeration among farm samples was more than market samples. Among three farms, enumeration of coliphages from A to C was decreased, respectively (Fig. 3).

Prevalence of Different Groups of F^+RNA Coliphages in Farm and Market

Molecular method (RT-PCR) showed that F^+RNA coliphages group I, II, and IV were detected in farm samples, whereas in market samples only group I was detected. In

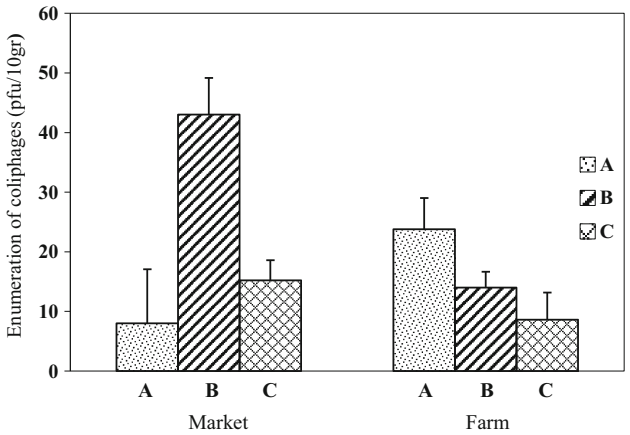

Fig. 3 Enumeration of coliphages in different sample collection sites

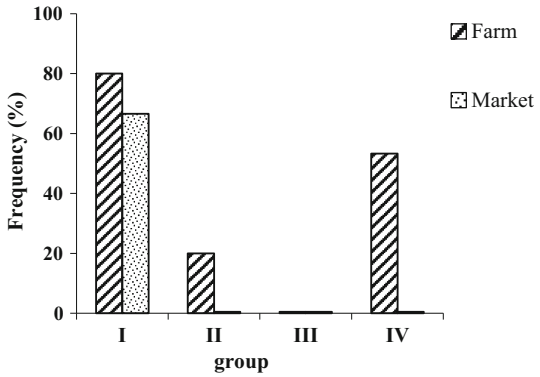

Fig. 4 Comparison of frequency distribution of different groups of F^+RNA coliphages in samples

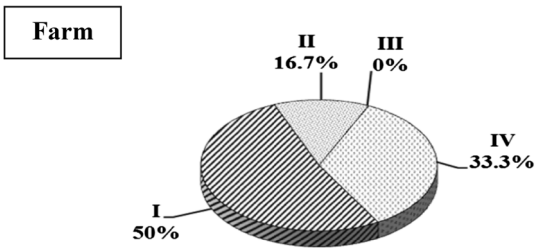

Fig. 5 Frequency distribution of different groups of F^+RNA coliphages in farm samples

Table 3 Number of F^+RNA coliphages positive samples (DAL and RT-PCR method)

Sample source	Number of samples	Positive number of samples				
		DAL assay	RT-PCR			
			FRNA coliphages group I	FRNA coliphages group II	FRNA coliphages group III	FRNA coliphages group IV
Farm						
A	5	5	5 (100 %)	3 (60 %)	0	5 (100 %)
B	5	4	4 (80 %)	0	0	2 (40 %)
C	5	3	3 (60 %)	1 (20 %)	0	1 (20 %)
Market						
A	5	2	2 (40 %)	0	0	0
B	5	5	5 (100 %)	0	0	0
C	5	3	3 (60 %)	0	0	0

Table 4 Sequencing of RT-PCR amplicons

Sample	Genotype	FRNA coliphages	Identity (%)
Farm	I	MS2(JF719743.1)	99
		F2(M31635.1)	96
	II	Ga(X03869.1)	96
		Ku1(AF227250.1)	96
		Th1(AB218930.1)	98
		Jp34(J04343.1)	95
		Bz13(FJ483839.1)	95
	IV	FI(FJ539133.1)	97
		MX1(AF059242.1)	99
Market	I	MS2(JF719743.1)	99

farm A and C, different groups of F^+RNA coliphages (I, II, and IV) were found. Also, F^+RNA coliphages group I was predominant among all groups of F^+RNA coliphages in farm and market samples (Fig. 4). Frequency distribution of F^+RNA coliphages group I was 50 %, followed by group IV 33.3 % and group II 16.7 % in the farm samples (Fig. 5; Table 3). This finding revealed that animal faces probably are the biggest source of pollution in these areas. Furthermore, few samples were positive for F^+RNA coliphages group II and no sample was detected for group III. Human fecal pollution was rarely detected on parsley and leek surface. Comparison of RT-PCR amplicons with NCBI database is shown in Table 4. These results confirmed the presence of different groups of F^+RNA coliphages.

Discussion

Nowadays, the role of fresh produce in transmission of enteric viruses is well understood. Various protocols have been published for extraction, concentration, and detection of viruses in foods (Dubois et al. 2002; Sair et al. 2002; Dubois et al. 2006; Butot et al. 2007; Dubois et al. 2007; Croci et al. 2008; Stals et al. 2012). However, only few methods have been applied successfully to detect viruses in naturally contaminated foods (Le Guyader et al. 2004; Williams 2005). Comparisons of different published methods are difficult because of various types of foods and viruses; thus, the application of a good indicator organism can be useful. Recently, F^+RNA coliphages have been suggested as a useful model and tracer in most researches carried out on viruses and fecal pollution of foodstuff (Sobsey et al. 2006; Yavarmanesh et al. 2010). These bacteriophages are not human pathogens, but they infect natural enteric bacteria in the mammalian's tracts and they are excreted in feces. In this study, F^+RNA coliphages as tracer to detect type of fecal contamination in parsley and leek were applied. Firstly, we compared two methods (PEG-precipitation and Ultracentrifugation) of extraction and concentration of MS2 from spiked mixed parsley and leek samples. Both methods were able to extract MS2 coliphages from spiked samples. However, the result of this study showed that the PEG-precipitation method was more efficient than Ultracentrifugation in which significant difference was observed between two recovery methods in the last spiked solution (10^6 pfu/ml). On the other hand, mean of coliphage number in the plants that were treated by sewage was reported 10^5 pfu/100 ml (Debartolomeis and Cabelli 1991).

The recovery efficiency obtained by PEG-precipitation method in this study was comparable with the previously published results (Dubois et al. 2002; Scherer et al. 2010; Sánchez et al. 2012; Summa et al. 2012). For example, in separate studies, Scherer et al. (2010) and Yazdi (2014) reported that MS2 coliphages recovery efficiency from spiked lettuce samples by the same PEG-precipitation method was 6–10 and 16 %, respectively (inoculation

concentration was 10^6 pfu/ml) which is more similar to our result (12 %) (Scherer et al. 2010; Yazdi 2014). Also, PEG-precipitation method was recommended as a useful protocol for virus extraction in previous studies because of the best repeatability of method and applicability for all food matrices (Summa et al. 2012).

The higher MS2 coliphage recovery obtained by PEG-precipitation may be explained by better performance of TGBE washing buffer. Similar buffers have been noted as effective washing solution in earlier researches. For example, Dubois et al. (2006) reported that buffer solution containing Tris–Glycine (pH 9.5) was more effective for MS2 coliphage recovery from lettuce; moreover, Bahraini (2011) demonstrated that the presence of beef extract in washing solution is important to extract viruses from the herbs.

In this study, due to the higher concentration of polyethylene glycol, virus precipitation time was less than other previous studies. It has already been shown that there is a relation between the shape of a virus and the PEG concentration in which higher concentration of PEG is required to precipitate small spherical viruses (e.g., F^+RNA coliphages) than the rod-shaped (Yamamoto et al. 1970; Vajda 1978). Moreover, changing the pH from alkaline to neutral is the main role in phage precipitation in condensed step due to the nature of phage capsid proteins and their isoelectric point (Sobsey and Meschke 2003). Capsid isoelectric pH of F^+RNA coliphages is usually in <7 then reducing of pH helps to precipitate phages in this step.

The vegetables and herbs have the potential to carry viral particles on their surface because of the type of fertilizer, irrigation, and lack of hygiene during harvest in farm or supply in markets. Therefore, fecal contamination in suspicious samples of parsley and leek was investigated to determine F^+RNA coliphages. The use of F^+RNA coliphages for evaluation of the hygiene of fresh food was reported (Doré et al. 2000; Endley et al. 2003).

In the present study, negative results for enumeration of coliphages can be related to inadequate accuracy for recovery and culture methods. On the other hand, there is no infective risk in 36.6 % of studied samples with coliphage number <10 pfu/10 g (infective dose of viruses is 10–100 virus particles).

The difference in contamination among market samples can be due to difference in the source of vegetables originated and different hygiene practices. Unlike market B, two other markets (A and C) were located in the city center with greater distance from the farms. Then, lower phage pollution as a high hygiene indicator has been seen in these markets.

The similar coliphage enumeration among farm samples may be due to the same situations of the sites. As well as the likeness of harvesting areas cause the similarity for coliphage enumeration of market A with farm C or market C with farm B. Since vegetables shelf life is short, the vegetables are quickly sold after harvesting and are not kept for the long time in the stores. Thus, the main source of viral pollution in vegetables like parsley and leek can be the farms.

In domestic storage, vegetables usually are kept cool to maintain their freshness. The low temperature may play a role in virus survival and its transmission to the human. It was illustrated that MS2 reduction in fresh produce (such as parsley, cabbage, lettuce, etc.) was <1 log after 50 days storage at 4 and 8 °C (Dawson et al. 2005). Similar observations were reported for virus survival in vegetables (celery, spinach, lettuce, and tomato) at 4 °C after irrigation with wastewater (Ward and Irving 1987). Therefore, the use of raw vegetables like parsley and leek after even one week of treatment with wastewater in farm will have high risk for consumers, because of cool storage at home. On the other hand, the average of air temperature in farms of Neyshabour city from April to July was usually lower than 28 °C which is relatively suitable for virus survival.

Detecting the type of fecal contamination in fresh produce by different groups of F^+RNA coliphages would be appropriate to control the virus contamination in food samples. For this purpose, we identified F^+RNA coliphages subgroups in parsley and leek samples. Results showed that only subgroup I was found in the market samples, while in farm samples all subgroups except III were observed. Samples of farm A had the most frequency for different subgroups of F^+RNA coliphages among all sites. Subgroup I of F^+RNA coliphages was the most frequent in farm or market samples, especially in farm A and market B. Previous studies revealed greater resistance of subgroup I under simulated natural conditions in water (Schaper et al. 2002). Therefore, the absence of subgroup III and little presence of subgroup II in our samples probably indicate lower resistance of these subgroups. However, the chance of pathogenicity with human virus in the absence of some subgroups of F^+RNA coliphages (II and III) will not decrease and more careful monitoring is needed in food samples.

Greater frequency of animal source genotypes found in samples is a result of irrigation with polluted water or fertilization with animal faces (hens and cows). Also, some animals like dogs and cats can be the main source of animal fecal contamination. Further study is needed to identify types of animal fecal pollution in fresh products and management or control of these contaminations.

Conclusion

The genotyping of F^+RNA coliphages by RT-PCR used in this study allowed characterization and identification of different types of fecal pollution in parsley and leek

samples and introduced these coliphages as a valid biomarker in microbial source tracking studies in food samples.

Compliance with Ethical Standards

Conflict of interest The authors (Dina Shahrampour, Masoud Yavarmanesh, Mohammad Bagher Habibi Najafi, and Mohebbat Mohebbi) confirm that this article content has no conflict of interest.

Human and Animal Rights and Informed Consent Also, this article does not contain any studies carried out with human or animal subjects.

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